

# **ROI Selection Markers**

And CRO Services for GeoMx® Digital Spatial Profiling

ROI Selection Markers are designed for use with the GeoMx® Digital Spatial Profiler (DSP) platform from NanoString. The GeoMx DSP platform offers high-plex spatial profiling of protein or RNA targets. Morphology markers are fluorescently labeled antibodies used to select biologically relevant regions of interest (ROIs) for spatially guided analysis of transcriptional and proteomic pathways. Morphology Marker Kits from NanoString broadly identify solid tumors, but researchers have requested additional cell- and disease-specific markers. Canopy Biosciences is a leader in CRO services for GeoMx DSP assays and has developed a catalog of ROI Selection Markers, which are cell- and disease-specific. Our catalog includes pre-qualified markers as a supplement to Morphology Marker Kits from NanoString, and includes critical targets in immunology, oncology, and neuroscience to enable ROI selection with greater precision.

# **Research Applications**

Our ROI Selection Markers are designed for a broad range of research applications in immunology, oncology, and neuroscience. The catalog includes markers relevant in recent literature and continues to expand by investigator request. This catalog focuses on key targets for immune cell profiling and immuno-oncology drug targets for:

- Cancer research aimed to profile molecular and cellular basis of cancer including tumor microenvironment, tumor evolution and response to treatment, and immune response to cancer
- Drug development and research including target selection and validation, preclinical toxicity studies, and pharmacodynamic studies

# Product Highlights

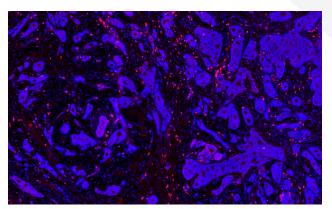
- Designed for GeoMx® Digital Spatial Profiling Assays
- Compatible with Morphology Marker Kits from NanoString
- Pre-validated according to guidelines from NanoString
- We can validate new targets to create a fully custom set of markers



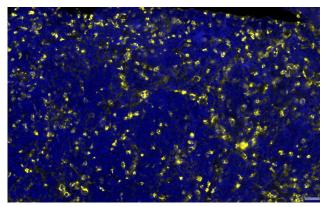
The GeoMx DSP system from NanoString is a highly sensitive and widely adopted method for ROI-guided analysis of transcriptional and proteomic pathways. As a preferred CRO partner for GeoMx DSP services, we are uniquely positioned to offer custom target validation for ROI selection. This catalog significantly expands the current availability of markers for use with FFPE and fresh frozen tissues:

- Immune Cell Profiling
- Immune Cell Activation Status
- Immuno-oncology Drug Targets
- Neuronal Cell Profiling
- Neuro-oncology Drug Targets
- Lung Oncology Targets

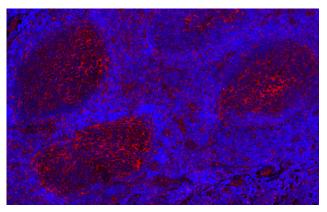




**Figure 1.** CD3 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.



**Figure 2.** CD68 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.



**Figure 3.** TIGIT expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

# Marker Catalog Development

Using the Morphology Markers validation guidelines from NanoString, we qualify and verify antibodies for use in FFPE or fresh frozen tissue that target your protein of interest. We can test and validate antibodies for virtually any antigen for any tissue type using antibodies conjugated to fluorophores compatible with the GeoMx DSP system. Select from our growing catalog of prevalidated ROI Selection Markers for targeting proteins in human tissue samples.

# **Custom ROI Selection** Markers

If your protein of interest is not available in this catalog, we can validate it for you. We continually test new targets for clients from any commercial vendor. Typically we focus on add-on markers to augment the off-theshelf kits provided by NanoString, but our capabilities also include validating fully custom morphology marker sets.

For more information, email us at info.canopy@bruker.com

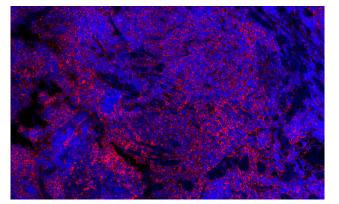


Figure 4. CD27 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

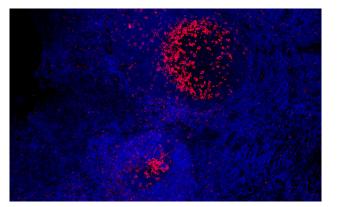


Figure 5. PD-1 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

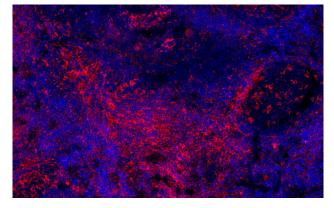


Figure 6. PD-L1 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

# **ROI** Selection Marker Validation

Canopy Biosciences follows the same testing approaches for gualification and verification of Morphology Markers presented by NanoString. Canopy Biosciences® ROI Selection Markers undergo extensive validation to ensure high quality datasets for precise analysis.

#### **Antibody Selection**

Antibodies with compatible fluorophores are carefully selected from commercial vendors. Markers must be IHC-validated and be available in the open channel along with the main imaging channels for GeoMx DSP.

#### Antibody Testing

We use NanoString's Morphology Marker Guidelines as the basis for our process to qualify and verify morphology markers for use in GeoMx DSP. Markers are tested in control tissues as a first pass to determine staining quality and compatibility with conditions for the transcriptomic analysis workflow.





#### **Qualification & Verification**

Target-specific positive tissue staining is verified by an experienced pathologist and dilution is optimized to assess specificity and reduce background. Antibodies are also tested in combination with other markers in a multiplexed assay to rule out spectral interference.

#### **Ongoing Evaluation**

We are continuously evaluating markers for their suitability for staining new tissue types and performing ongoing optimization. Antibodies undergo testing on multiple tissues and are successfully used for ROI segmentation.

ONGOING **EVALUATION** 

# **ROI Selection Marker List**

Immune Cell Profiling: Critical markers to identify immune cell types, including T cells, B cells, macrophages, and NK cells. Additional markers for deeper profiling of immune cells, including subtyping T cells.

Immune Cell Profiling			
Marker	Description		
CD3	Key marker of T-cells with a critical role in T-cell mediated responses		
CD4	Key marker of helper T-cells with a critical role in adaptive immune response		
CD11c	Key marker of dendritic cells, macrophages, and neutrophils		
CD8	Key marker of cytotoxic T-cells with a critical role in targeted cell killing		
CD45	Key marker of immune cells with a critical role in T-cell activation		
CD68	Key marker of monocytes and macrophages		
FoxP3	Key marker of regulatory T-cells with a critical role in cell development		
Granzyme B	Key marker of cytotoxic T-cells and NK cells with a critical role in apoptosis		

Immuno-oncology Drug Targets: Critical drug targets in development in immunooncology, including many immune checkpoint molecules. Drug targets have the potential to enhance anti-cancer immune responses.

	Immuno-oncol
Marker	Description
B7-H4	Inhibitory receptor involved in T
CTLA-4	Inhibitory receptor involved in T
IDO	Inhibitory enzyme involved in tr
LAG-3	Inhibitory receptor involved in
OX40L	Inhibitory receptor involved in [
PD-1	Inhibitory receptor involved in
TIGIT	Inhibitory receptor involved in T
TIM-3	Inhibitory receptor involved in i

Immune Cell Activation: Critical checkpoint molecules that modulate T cell activation. Key markers of T cell activation mediate the progression of immune response.

Immune Cell Activation			
Marker	Description		
CD27	Key marker of memory B cells with a critical role in T-cell proliferation		
CD28	Key marker of activated T-cells with a critical role in T-cell proliferation		
OX40	Key marker of activated T-cells, also expressed on NK cells, NKT cells, neutrophils		
PD-L1	Key marker of activated T-cells and immune checkpoint inhibitor		



#### logy Drug Targets

- T-cell activation and cytokine production
- T-cell activation in early immune response
- ryptophan and interleukin production
- T-cell activation and effector functions
- DNA-binding transcription factor activity
- T-cell activation and apoptosis
- T-cell activation and interleukin production
- interleukin and interferon production

# **ROI** Selection Marker List

Neuronal Cell Profiling: Critical markers to identify neuronal cell types including microglia, macrophages, and others. Additional markers for deeper profiling of cells involved in regulating neurotransmitter synthesis and ion transport.

Neuronal Cell Profiling			
Marker	Description		
ChAT	Key marker of cholinergic neurons with a critical role in acetylcholine synthesis		
DAT	Key marker of neuronal synapse with a critical role in dopamine transport		
GAD67	Neuronal cell marker with a critical role in GABA synthesis		
GFAP	Key marker of astrocytes with a critical role in cell development		
MBP	Neuronal cell marker with a critical role in myelin formation and stabilization		
Nestin	Key neuronal cell marker with a critical role in axon growth		
NeuN	Neuronal cell marker with a critical role in pre-mRNA alternative splicing		
Synaptophysin	Neuronal cell marker with a key role in synaptic plasticity		
ТН	Neuronal cell marker with a critical role in dopamine synthesis		
ТРН	Neuronal cell marker with a critical role in seratonin synthesis		
VGLUT	Neuronal cell marker with a critical role in glutamate ion transport		

Neuro-oncology Drug Targets: Critical drug targets in development and prognostic markers in neuro-oncology. Drug targets have the potential to enhance anti-cancer immune responses, while prognostic markers may help predict disease course.

	Neuro-onco
Marker	Description
EGFR	Cell surface receptor with a key
FOXG1	Transcriptional regulator with a
IDH	Mitochondrial enzyme with a k
L1CAM	Cell adhesion protein with a ke
Olig2	Transcriptional regulator with a
p53	Transcriptional regulator and to
PTEN	Transcriptional regulator and tu
Sox2	Transcriptional regulator with a



#### ology Drug Targets

				1.6
У	role	in	cell	proliferation

- a key role in neuronal cell differentiation
- key role in the generation of NADPH
- ey role in migration and cell-cell communication
- a key role in cell differentiation
- tumor suppressor with a key role in cell survival
- tumor suppressor with a key role in cell survival
- a key role in cell differentation

# **ROI Selection Marker List**

Lung Oncology Targets: Critical prognostic markers and drug targets in lung oncology. Drug targets have the potential to enhance anti-cancer immune responses, while prognostic markers may help predict disease course.

Lung Oncology Targets				
Marker	Description			
ALK	Cell surface receptor and key marker in non-small cell lung cancer			
INSM1	Transcriptional regulator and key marker in neuroendocrine tumors			
MET	Cell surface receptor with a key role in cell proliferation			
Napsin	Intracellular proteinase and key marker in lung adenocarcinoma			
p40	Transcriptional regulator and key marker in squamous cell carcinoma			
p63	Transcriptional regulator and key marker in small cell carcinoma			
ROS1	Cell surface receptor and key marker in non-small cell lung cancer			
TTF-1	Transcriptional regulator and key marker in lung adenocarcinoma			

# About Us

Canopy Biosciences is a global provider of products and services for accelerating multi-omics research. We acquire novel scientific instruments and offers CRO services for:

- ChipCytometry™
- Histopathology/IHC
- FISH analysis
- Single-cell and bulk RNAseq
- nCounter Analysis System
- GeoMx Digital Spatial Profiler

As a CLIA-certified laboratory, we are equipped to provide support for preclinical and clinical trial studies.

For custom projects, email us at info.canopy@bruker.com

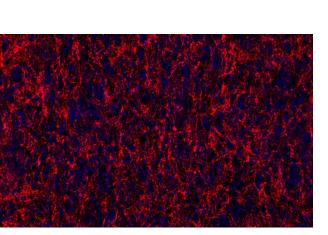


Figure 7. MPB expression in human glioma tissue using Canopy Biosciences® ROI Selection Markers.

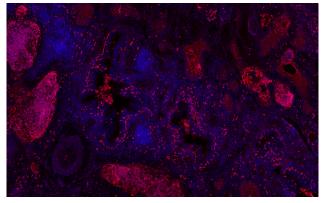


Figure 8. p63 expression in human lung tissue using Canopy Biosciences® ROI Selection Markers.

### Contact Us for Custom Markers

If our catalog of ROI Selection Markers does not include your protein of interest, contact us for custom marker validation.



#### To learn more, visit CanopyBiosciences.com/ or email us hello.canopy@bruker.com

Canopy Biosciences 4340 Duncan Avenue Suite 220 Saint Louis, Missouri 63110

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